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14. ABSTRACT A major goal in cancer immunotherapy is to generate an effective anti-tumor immune response. Adoptive immunotherapy involvesstimulation of tumor-specific T cells, ex vivo (outside the body), followed by transfer of expanded numbers of activated T cells back intopatients. While adoptive immunotherapy holds promise as a treatment for cancer, development of adoptive immunotherapy has beenimpeded by the lack of a reproducible and economically viable method for generating therapeutic numbers of antigen-specific CTL. Thework proposed in this application will enable advances in adoptive immunotherapy. Most prostate cancers express prostate specificmolecules. These molecules, including PSA and PMSA, can serve as potential targets for immune-based treatments. Studies on immunerecognition of these molecules have already identified potential target regions within these proteins and are the basis of a variety ofdifferent experimental immunotherapies for treatment of prostate cancer. In this study we propose to study the ability to use HLA-Ig basedaAPC as a viable method for induction, expansion and activation of prostate specific T cells for immunotherapy for prostate cancer. Thesestudies will serve as precursor ones for induction and expansion of prostate specific CTL from patients with disease for initiation ofadoptive immunotherapy phase I clinical studies.

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Introduction:

Since the last report our focus has been on induction of anti-PSA-3A and PSA-1 prostate-specific CTL. Previously we have development the animal model, as described in the original Specific Aim #2, to test the in vivo efficacy of aAPC induced prostate cancer specific CTL. Once we growth of anti-PSA-3A and PSA-1 prostate-specific CTL in place we will be able to test their in vivo efficacy as described in Specific Aim #2.

Background: An Overview of Adoptive Immunotherapy

A major goal in the field of immunotherapy is to generate an effective cell-mediated anti-tumor immune response. Adoptive immunotherapy involves induction and expansion of antigen-specific T cells, *ex vivo*, followed by transfer of autologous antigen-specific T cells back into patients.

It has been demonstrated in both animal and human models that *ex vivo* expanded MHC Class I-restricted CD8+ cytotoxic T-lymphocytes (CTL) are able to kill virus-infected cells and tumor cells. While antigen-specific, antiviral CTL were first evaluated in humans by investigators at the Fred Hutchinson Cancer Research Institute [1, 2] who administered *ex vivo* expanded cytomegalovirus (CMV)-specific CTL clones as prophylaxis for CMV disease, Rosenberg's group [3] at the National Cancer Institute was first to successfully expand autologous tumor-specific T cells, referred to as tumor-infiltrating lymphocytes (TIL), *ex vivo*, and re-infuse them into melanoma patients together with IL-2 to help maintain both numbers and function of the re-infused cells. In a modest percentage of responding patients, they were able to show that re-infused TIL had trafficked back to tumor sites and directly induced tumor shrinkage, *in vivo*. Recent work has shown that adoptive transfer, in patients with metastatic melanoma, following a non-myeloablative conditioning regimen resulted in regression of the patients' metastatic melanoma as well as the onset of autoimmune melanocyte destruction [4]. More recent studies also show that adoptively transferred CTL can survive in patients with metastatic melanoma and track to the site of the tumor [5]. All these studies highlight the importance of exploring adoptive immunotherapy as a promising means to generate an effective cell-mediated anti-tumor/viral immune response for expanded clinical evaluation.

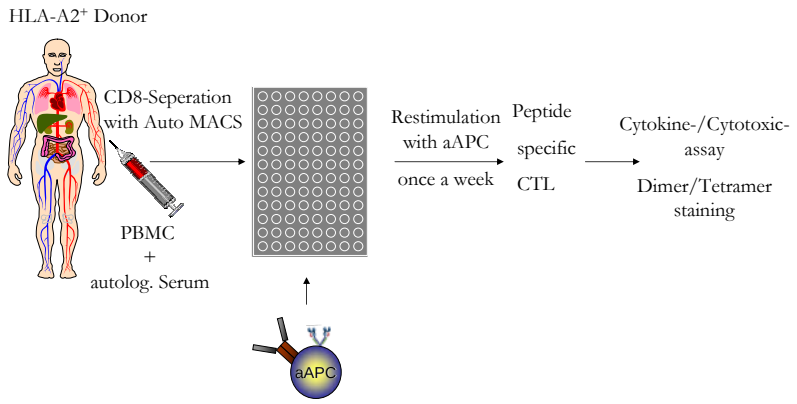
The development of an artificial Antigen-Presenting Cell (aAPC) has opened the gateway for *ex vivo* stimulation and expansion of tumor-specific T cells to clinically relevant numbers. Initially, June and colleagues developed approaches for non-specific expansion of CTL derived either from TIL-cultures or tetramer-based sorting for enrichment of antigen-specific CTL. By coupling beads to anti-CD3 and anti-CD28 mAbs, they have been able to expand CD4+ T cells. However, this non-specific system is limited in two ways. First, such anti-CD3/anti-CD28 beads failed to support long-term growth of CD8+ CTL, even when T cell growth factor (e.g. IL-2) was added. Second, it was not possible to maintain the antigen-specificity during expansion [6, 7], both significant requirements for induction and expansion of tumor-specific T cells to clinically relevant quantities. Our preliminary data demonstrate that an artificial Antigen Presenting Cell (aAPC), made by coupling HLA A2-Ig and anti-CD28 to beads, can

reliably induce and expand antigen-specific CTL from healthy donors. This approach promises to be a facile, cost-effective, and excellent alternative to the more time consuming and expensive dendritic cell based expansion. In our preliminary data, which supported funding of the grant, we showed that this approach can be used to expand multiple clinically relevant CTL populations including CTL specific for CMV or for melanoma antigens. In the current application we proposed to demonstrate functional efficacy of HLA-Ig complexes conjugated to beads as artificial Antigen Presenting Cells (aAPC) for inducing and expanding anti-PSA-3A and PSA-1 prostate-specific CTL. The specific aims are to *1) optimize aAPC structure and duration of stimulation, and 2) analyze the in vivo function of aAPC-induced CTL.*

Body:

For evaluating the efficacy of HLA-A2-Ig based artificial Antigen Presenting Complexes (aAPC) approach for treatment of prostate, we proposed to study in vitro expansion of CTL from blood and looked at the response to several candidate antigens. We initially focused on our goals, as identified in the Specific Aims of the application, on induction of anti-PSA-3A and PSA-1 prostate-specific CTL.

Schematic for Induction and Expansion of peptide-specific CTL using aAPC



CD8+ T-cells were isolated from healthy donors and co-cultured with aAPC as described in the schematic above for a 4-10 week period. PBMCs were obtained from healthy donors by Ficoll centrifugation and separated for CD8+ t-cells. After separation CD8+ T-cells were cocultured with peptide pulsed aAPCs and harvested after one week. The old beads were removed; cells were counted and restimulated in 96 well plates with fresh aAPC. This procedure was normally performed until cell number was reduced to less than $5x10^5$ cells.

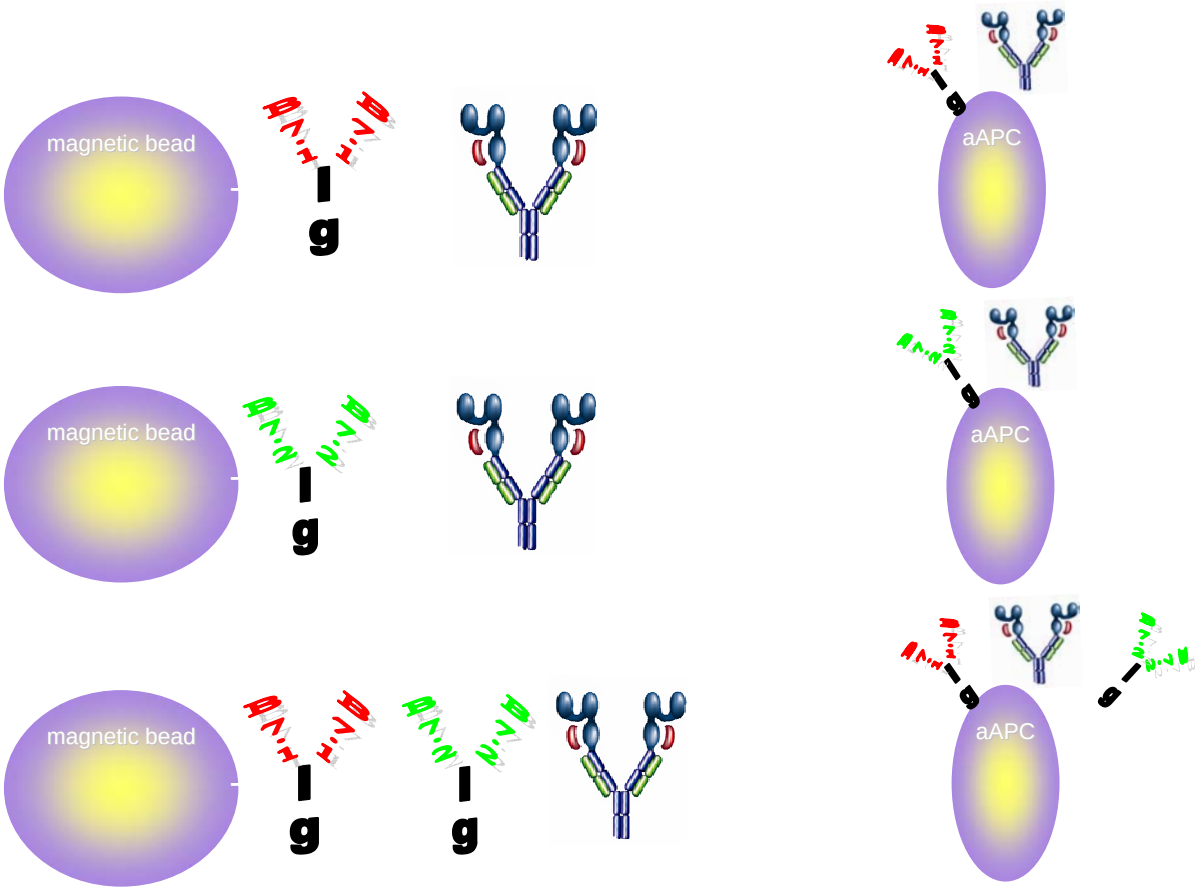
We performed the stimulation as shown in the schematic for 8 donors with PSA-3A and PSA-1 peptide pulsed aAPC. As a positive control we additionally pulsed aAPC with Mart-1 peptide. After 4 weeks no specific CTL could be seen for the experimental

peptides whereas the culture co-incubated with the Mart-1 pulsed aAPC showed specific cells in a tetramer stain after 2 weeks. Further investigation was restricted by the limited CTL number obtained in the aAPC stimulated culture.

Since the approach did not work with the current aAPC formulation, we have started to develop second generation aAPC formulations as proposed in the original application. B7-1 (CD80) and B7-2(CD86) on dendritic cells are the natural ligands to CD28 on T-cells. In our first generation of aAPC beads, we used beads loaded with the chimeric fusion protein along with an anti-CD28 antibody to co-activate T-cells. We postulated that the natural ligand might have a higher activation activity than the antibody against CD28. Therefore we created a new generation of beads coated with B7-1, B7-2 or B7-1/2 as a co-stimulatory molecule instead of the anti-CD28 antibody.

Three different types of beads were generated as described below and shown schematically:

Bead with HLA A2-IgG1-dimer	+B7-1-IgG
Bead with HLA A2-IgG1 dimer	+B7-2 IgG
Bead with HLA A2-IgG1-dimer	+B7-1IgG and B7-2 IgG

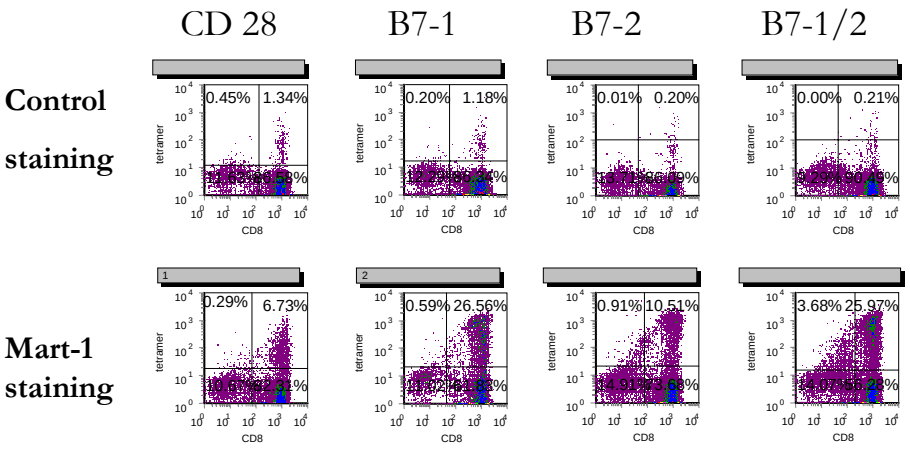


To further enhance the activation that might be very small because the antigens are known to be weak antigens IL-2 was added to the cultures in a concentration of 100U/ml of culture medium.

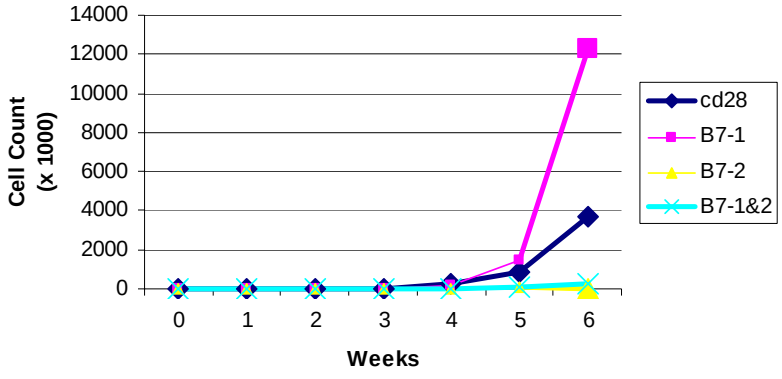
To investigate this new system we compared the stimulation of the new beads with the one of the beads loaded with the CD28AB in the Mart-1 system. Mart-1 is a relatively strong antigen and was shown in a recent paper of our group to work well for induction of functional CTL.

We found that for 2 out of 3 donors the response to the stimulation was much stronger for the B7-1 coated bead than for the anti-CD28 coated one regarding specificity in tetramer staining (see figure below). A much higher proliferation was also found for the stimulation with the B7-1 bead for the one donor with the higher specificity with the anti-CD28 loaded aAPC stimulated cells (see below).

B7-1 based aAPC stimulate a higher percent antigen-specific CTL than anti-CD28 based aAPC



2nd Generation B7.1/HLA A2-Ig based aAPC expand antigen-specific CTL better than anti-CD28 aAPC



Given that the anti-CD28 loaded beads were made from a different batch of HLA A2-Ig dimer than the B7-1, B7-2 and B7-1/2 aAPC, we made new beads with one batch of HLA-A2-Ig dimer to explore the effects of co-stimulation. Three new beads were

made: One was loaded with the HLA A2-IgG dimer and the anti-CD28 antibody, one with the HLA A2-IgG dimer and the B7-1-Ig and one with the HLA A2-IgG dimer and an IgG2a isotype spacer molecule. All the HLA A2-IgG dimer molecules came from the same sample and the co-stimulatory/spacer molecules were added in an equal molar weight ratio. We are currently testing the new batches of beads on expansion of Mart-1 specific cells and form there will test their ability to support expansion of anti-PSA-3A and PSA-1 prostate-specific CTL.

Bulleted List of Key Accomplishments:

1) Development of two model in vivo prostate tumor growth in SCID mice
    a. Using LNCaP cells
    b. PC-3
2) Obtained IRB approvals
3) Obtained material for starting work with human blood products including various different accessory molecules detailed in Specific Aim #1.
4) Tested several different donors for expansion of anti-PSA-3A and anti-PSA1 specific CTL. None of the donors showed expansion of specific CTL.
5) Prepared new chimeric proteins for second generation aAPC based on B7.1 and B7.2 mediated stimulation.
6) Tested second generation aAPC in systems for expansion of Mart-1 specific CTL from multiple donors.

Summary:

We have highlighted studies that demonstrate the importance of exploring adoptive immunotherapy as a logical method for treatment of prostate cancer. As discussed, the ability to produce clinically relevant amounts of antigen specific T-cells for adoptive transfer has been limited. Our method represents a novel approach, whereby the generation and expansion of antigen-specific CTL starting from low precursor frequency to significant amounts is facile and cost effective. Furthermore, the enhanced stimulatory capacity and specificity of the dimeric peptide-HLA-Ig constructs, when formulated with co-stimulatory molecules will provide a platform for a straightforward and reproducible cell culture process resulting in CTL activation and expansion useful for therapeutic applications.

To summarize, the proposed experiments will continue to compare aAPC-mediated and DC-mediated T cell induction, expansion and differentiation of antigen-specific CTL. In this application, we proposed to evaluate varying parameters essential to optimize aAPC function. These include altering the ratios of signal 1 to signal 2 and the types of signal 2 on the aAPC. After establishing the use of these new beads for expanding Mart-1specific CTL, we will then look at the ability of the second generation aAPC to expand prostate-specific CTL. These results will permit us to move effectively into evaluation of the *in vivo* efficacy of aAPC-expanded prostate-specific CTL.

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